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PATENT
Docket No. 286002020022
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Margaret P. Drosos

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Carol Clayberger *et al.*

Serial No.: 08/222,851

Filing Date: 5 April 1994

For: CYTOTOXIC T-CELL LYMPHOCYTE ("CTL") ACTIVITY REGULATION BY CLASS I MHC PEPTIDES

Examiner: T. Cunningham

Group Art Unit: 1813

DECLARATION OF CAROL CLAYBERGER PURSUANT TO 37 C.F.R § 1.131

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, Carol Clayberger, declare as follows:

1. I am one of the inventors named on US Serial No. 08/222,851, filed 05 April 1994, which is a CIP of US Serial No. 07/844,716, filed 02 March 1992, which is a CIP of US Serial No. 07/755,584, filed 03 September 1991, which is a Continuation of US Serial No. 07/672,147 filed 19 March 1991, which is a CIP of US Serial No. 07/008,846, filed 30 January 1987.

2. I have read the Office Action mailed 25 September 1995 for the above referenced application.

3. At page 4, Section 19B of the Office Action, the Examiner has stated that the application only demonstrates inhibition of CTL's which have shared MHC specificity with the administered peptide. The Examiner has cited page 26 of the specification where it is stated that "the results in table 2 indicate that only when the CTL's target cells share A2 specificity do the A2 peptides provide inhibition" to support this position. The Examiner has concluded that an administered peptide "would be expected only to inhibit that portion of host CTL response directed to that particular HLA."

The Examiner is incorrect in his assertion that the inhibition provided by the peptides used in the present method are HLA specific and the application only shows MHC specific inhibition. The results noted by the Examiner were obtained when fragments of polymorphic regions of the α_1 or α_2 domains of certain MHC class I molecules were used. The results noted by the Examiner did indeed demonstrate that these fragments only blocked lysis by CTL's specific for the MHC molecule from which the peptide was derived. Because of the allele specificity of these observations, we turned our attention to less polymorphic regions of the HLA class I molecules, and in particular residues 75-84 of the α_1 domain.

Of the HLA-B alleles chosen, HLA-B7.75-84 (RESLRNLRGY) and HLA-B2702.75-84 (RENLRIALRY) were found to inhibit one or more of the following: 1) CTL proliferation *in vitro* and *in vivo*, 2) CTL differentiation *in vitro* and *in vivo*, 3) CTL mediated lysis *in vitro* and *in vivo*, and 4) allograft rejection *in vivo*. Unlike the earlier work, the inhibitory activities of peptides comprising residues 75-84 were not allele specific. The result which demonstrate the non-MHC specific inhibition of CTL activity can be found at least as follows:

i) At page 40, line 3 of the application, it is disclosed that "the peptide corresponding to residues 75-84 of HLA-B2702 blocked all class I specific CTL responses . . .."

ii) At page 47 of the specification, the effects obtained from *in vivo* administration of human HLA peptides to rats on subsequent allograft challenges *in vivo* and *in vitro* are provided. At line 24, it is disclosed that "splenocytes obtained from animals treated with the B7.75-84 or B2702.75-84 peptide 7 or 10 days prior to splenectomy showed an 8-10 fold decrease in the precursor frequency of Lew specific CTL."

iii) Additional *in vivo* results demonstrating that the inhibition of CTL's is not MHC specific are provided at pages 49-50 of the specification. In the studies summarized, both the B7.75-84 and B2702.75-84 peptide provided a level of protection against allograft rejection greater than that observed with controls.

iv) In addition to the data provided in the specification, attached hereto as Appendix A is a copy of Nisco *et al.*, J. Immunol. 152:3786-3792 (1994), of which I am a co-author. The results provided in Nisco demonstrated that the human HLA-B2702.75-84 and human HLA-B7.75-84 peptides 1) inhibited the differentiation of allospecific CTLs *ex vivo*, 2) inhibited the accumulation of lymphocytes in lymph nodes after challenge with allogenic cells, and 3) indefinitely inhibited cardiac allograft rejection. Since the one known rat MHC class I molecule (RT1A) contains only a 70% identity in amino acid sequence with HLA-B7.75-84 and only a 50% homology with HLA-B2702.75-84, the inhibitory effect obtained was clearly not MHC specific as asserted by the Examiner.

4. At page 5 section 19C of the Office Action, the Examiner has stated that "it would be unpredictable which peptide species would be capable of multiallele blocking without testing of different peptide species on a case-by-case basis."

There are presently six known sequences corresponding to residues 75-84 of human HLA-B, namely B2702, B38, B7, B62, B2705, and Bw46. These are reviewed in Wan, et al. J. Immunol. 137:3671 (1986) and Clayberger *et al.* Transplantation Pro. 25:477-478 (1993). Of these six known sequences, the application provides evidence that peptides containing the B2702 and B7 sequences provide multiallele blocking whereas B2705 is not effective. These results 1) provide guidance as to which sequences work and which do not, 2) demonstrate that some sequences provide multiallele blocking whereas others do not, and 3) demonstrate that one skilled in the art could test the other HLA-B alleles, without undue experimentation, for multiallele blocking activity. Accordingly, although not predictable, one of skill in the art can generate and test fragments containing residues 75-84 of the other HLA-B alleles for multiallele bl cking without undue experimentation.

5. At page 6, Section 19D of the Office Action, the Examiner has stated that "it would be unpredictable which mutations of the HLA-B 75-85 sequence would retain the critical functional property of being able to inhibit CTL activity because such mutations would be expected to affect functional binding of the peptide to T-cell receptor or accessory molecules."

At page 40, line 6 of the application, experiments are disclosed in which an amino acid in the HLA-B2702.75-84 sequence was altered and the effect the alteration had on CTL inhibitory activity was determined. The generation and testing of the variants described was accomplished using routine experimentation. Specifically, because the test peptides are short sequences, automated synthesis methods were employed to generate the peptides with the altered sequences. Such synthesis procedures could be used to generate a peptide corresponding to any of the possible amino acid substitutions for such a sequence. Although the effects that any particular amino acid substitution would have on the CTL inhibitory activity present in the parent molecule is not predictable, a skilled artisan can readily generate any particular altered sequence and test the altered peptide without undue experimentation.

6. At page 7, Section 19F of the Office Action, the Examiner has stated that "one skilled in the art would not reasonably expect to be able to use the claimed compounds, peptide compounds, and peptide conjugates because it would be unpredictable and require undue experimentation to demonstrate whether such peptide-based products would exert functionally useful effects on CTL responses *in vivo*." The Examiner has reasoned that immunogenicity of the peptides, degradation and host clearance would be expected to prevent the claimed activity.

In opposite to the Examiners position, the specification, as well as the attached Nisco article, provide evidence that the peptides are effective *in vivo* in inhibiting CTL activity. The immunogenic and clearance barriers suggested by the Examiner were not found to be present at a sufficient to abolish activity even when a peptide consisting of a human sequence was administered to rats.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on informati n and belief are believed to by true; and further that these statements were made with the knowledge that willful false statements and the like so made are

punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

4/15/96
Date

Carol Clayberger